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Short Communication

Combination Effect of Ciprofloxacin and Gentamicin against Clinical Isolates of *Salmonella enterica* Serovar Typhi with Reduced Susceptibility to Ciprofloxacin

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SUMMARY: The present study evaluated the in vitro efficacy of ciprofloxacin (CPFX) in combination with gentamicin (GM) using agar dilution checkerboard method against six blood culture isolates of *Salmonella enterica* serovar Typhi with CPFX minimum inhibitory concentration (MIC) values of 0.75 - 1.25 μg/ml and GM MIC values of 0.75 - 2 μg/ml. When used in combination, the fractional inhibitory concentration (FIC) values of CPFX and GM for the isolates ranged from 0.008 - 0.032 μg/ml and 0.1 - 0.2 μg/ml, respectively. The range of the FIC index from 0.121 - 0.216 indicated the synergistic effect between CPFX and GM for all the isolates. The time-kill method, which showed a 2.64 $\log_{10}$ decrease in CFU/ml between the combination and its more active compound, also established synergism between CPFX and GM against one isolate employed in the method. These results may be helpful in making clinical decisions in the treatment of enteric fever due to the infection of multidrug resistant *S. enterica* serovar Typhi.

The emergence of the multidrug resistant (MDR) *Salmonella enterica* serovar Typhi isolates showing resistance to chloramphenicol, ampicillin, and cotrimoxazole has been reported to make treatment difficult and in turn resulted in an epidemic situation (1). An enteric fever epidemic due to the infection of MDR *S. enterica* serovar Typhi having resistance to ciprofloxacin (CPFX), a fluoroquinolone introduced in the treatment of enteric fever over a decade ago, has also been reported (2). Gentamicin (GM), an aminoglycoside that is not commonly used in enteric fever, was found effective against *S. enterica* serovar Typhi infection (3). The enhancement of bactericidal activity of GM in combination with other antibiotics has been reported against enterococci (4). Huovinen et al. (5) established a synergy between CPFX and trimethoprim against *Escherichia coli*, staphylococci, and enterococci. However, the combined effect of CPFX and GM has not been reported so far against *S. enterica* serovar Typhi. Therefore, in this communication, we studied the activity of CPFX in combination with GM against *S. enterica* serovar Typhi isolates in order to evaluate the GM-mediated suppression of CPFX-resistance.

The present study was conducted using six blood culture isolates of *S. enterica* serovar Typhi obtained during 2000 - 2001 at the Calcutta School of Tropical Medicine. These isolates showed high minimum inhibitory concentration (MIC) values (0.75 - 1.25 μg/ml) to CPFX.

The isolates were tested in order to determine the MIC values of GM using the agar dilution method (6). For this purpose, a number of MHA plates containing different concentrations of GM ranging from 0.25 - 5 μg/ml were prepared. Each plate was divided into six equal sectors for spot inoculation using an inoculum of approximately 10^4 CFU/spot. The plates were then incubated at 37°C for 24 h.

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The combined effect of GM and CPFX against the isolates was studied using the checkerboard agar dilution method (7) involving eight different combinations of antibiotic concentrations ranging from 0.00625 - 0.8 μg/ml and 0.004 - 0.512 μg/ml, respectively, for GM and CPFX. This method also utilized an inoculum of approximately 10^4 CFU/spot, and the inoculated plates were incubated for 24 h at 37°C. The highest dilution of the antibiotic combination that inhibits the visible growth of the test organisms was regarded as the fractional inhibitory concentration (FIC) value. Synergy was defined as an FIC index ≤0.5, addition as an FIC index 0.5 - 4 and antagonism as an FIC index >4 (4).

Time-kill study was carried out for one (D1/01) of the isolates used in the checkerboard agar dilution method in order to evaluate the effect of GM and CPFX using 0.25 μg/ml for each, in combination and alone. An overnight-grown bacterial suspension was diluted to approximately 5×10^5 CFU/ml in fresh nutrient broth and incubated at 37°C. The samples removed at 0, 3, 6, and 24 h were diluted and plated on MHA in order to determine viable cell counts. Synergism was defined as a ≥2 $\log_{10}$ decrease in CFU/ml between the combination and its most active compound after 24 h of incubation (4).

The results of the study of the combined antimicrobial activity are shown in Table 1. The MIC range of GM from 0.75 - 2 μg/ml against the *S. enterica* serovar Typhi isolates, which were clinically unresponsive to CPFX showing MICs of 0.75 - 1.25 μg/ml, indicated their sensitivity to GM. CPFX, in combination with GM, showed FICs of 0.008 - 0.032 μg/ml. FICs of GM for the isolates ranged from 0.1 - 0.2 μg/ml. For all the isolates the FIC index was ≤0.5, and ranged from 0.121 - 0.216.

The results of the time-kill studies are shown in Fig. 1. The combination of CPFX and GM was inhibitory after 3 h of incubation. After incubation for 24 h the bacterial cell count was reduced by 2.64 $\log_{10}$ CFU/ml. Neither of the drugs showed bactericidal activity against *S. enterica* serovar Typhi at a concentration of 0.25 μg/ml of CPFX or of GM, when

Table 1. Results of the combined effect of ciprofloxacin (CPFX) and gentamicin (GM) against *S. enterica* serovar Typhi isolates

Isolates	MIC (μg/ml)		FIC (μg/ml)		FIC index
	CPFX	GM	CPFX	GM	
B41	1.00	1.00	0.016	0.2	0.216
D1/01	1.25	1.25	0.016	0.2	0.172
2/2K	0.75	2.00	0.016	0.2	0.121
S1/01	1.00	1.25	0.008	0.2	0.168
B69	0.75	1.25	0.032	0.1	0.122
B/219	1.25	0.75	0.032	0.1	0.159

MIC, Minimum inhibitory concentration; FIC, Fractional inhibitory concentration.

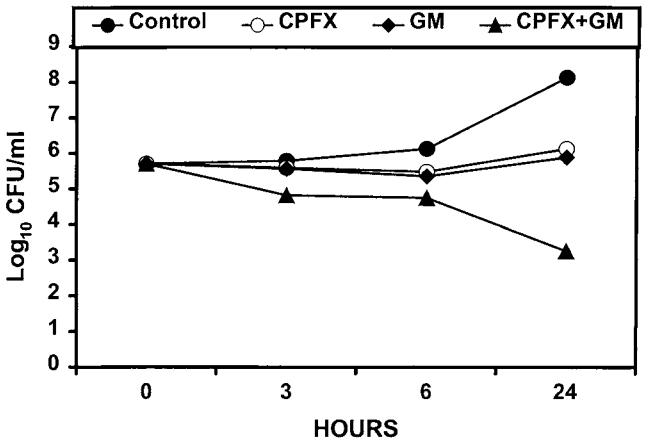

Fig. 1. Time-kill curves for a clinical isolate of *S. enterica* serovar Typhi using ciprofloxacin (CPFX) and gentamicin (GM). The control was antibiotic-free medium.

used alone.

The synergistic effects of aminoglycosides with other antibiotics has been reported against clinical bacterial isolates (8,9). Huovinen et al. (5), Moody et al. (10), and Neu (11) established synergism between fluoroquinolones and non-fluoroquinolones. Combination therapy using fluoroquinolone derivatives and aminoglycosides to treat *S. enterica* serovar Typhi infection has been suggested (12). Information on the in vitro interaction between CPFX and GM is still lacking against any clinical isolate of bacteria including *S. enterica* serovar Typhi. However, in the present study, the interaction between CPFX and GM was found to be synergistic against *S. enterica* serovar Typhi following two different methods. Additive and antagonistic activities were not found in our studies. Thus the combination of CPFX and GM may be a potential clinical regimen in combating the CPFX-resistance of *S. enterica* serovar Typhi.

In the treatment of enteric fever, GM is avoided considering its low intracellular concentration and the survival of *S. enterica* serovar Typhi inside macrophages. However, an earlier therapeutic trial by Madan et al. (12) claimed a favorable response using quinolone derivatives and aminoglycoside antibiotics in combination. This result may be due to the fact that the combination of these antibiotics kept in check the extracellular bacteremia and septicemia, keeping the infection load to a minimum level restricted to already infected macrophages. This enables the gradually mounting protective cell-mediated immunity to combat effectively the residual bacilli inside macrophages. A further therapeutic trial to corroborate the present view will establish a low cost therapy for MDR *S. enterica* serovar Typhi for the people of developing countries, keeping at bay the need for costly third and fourth generation cephalosporins.

REFERENCES

1. Anand, A. C., Kataria, V. K., Sing, W. and Chatterjee, S. K. (1990): Epidemic multiresistant enteric fever in eastern India. Lancet, 335, 362.
2. Murdoch, D. A., Banatvala, N. A., Bone, A., Shoismatulloev, B. I., Ward, L. R. and Threlfall, E. J. (1998): Epidemic ciprofloxacin resistant *Salmonella typhi* in Tajikistan. Lancet, 351, 339.
3. Adhikari, M. R. P. and Baliga, S. (2002): Ciprofloxacin resistant typhoid with incomplete response to cefotaxime. J. Assoc. Physicians India, 50, 428-429.
4. Leclercq, R., Bingen, E., Su, Q. H., Lambert-Zechovski, N., Courvalin, P. and Duval, J. (1991): Effects of combinations of β-lactams, daptomycin, gentamicin and glycopeptides against glycopeptide-resistant enterococci. Antimicrob. Agents Chemther., 35, 92-98.
5. Huovinen, P., Wolfson, J. S. and Hooper, D. C. (1992): Synergism of trimethoprim and ciprofloxacin against clinical bacterial isolates. Eur. J. Clin. Microbiol. Infect. Dis., 11, 255-257.
6. Miles, R. S. and Amyes, S. G. B. (1986): Laboratory control of antimicrobial therapy. p. 151-178. *In* J. G. Collee, A. G. Fraser, B. P. Marmion and A. Simmons (eds), Mackie and McCartny Practical Medical Microbiology. 14th ed. Churchill Livingstone, New York.
7. Krogstad, D. J. and Moellering, R. C. (1980): Combinations of antibiotics, mechanisms of interaction against bacteria. p. 298-341. *In* V. Lorian (ed), Antibiotics in Laboratory Medicine. The Williams and Wilkins Co., Baltimore.
8. Moellering, R. C., Wennersten, J. C. and Weinberg, A. N. (1971): Synergy of penicillin and gentamycin against enterococci. J. Infect. Dis., 124 (Suppl.), S207-S209.
9. Yu, P. K. W., Washington, J. A. and Hermans, P. E. (1983): Bactericidal and synergistic activity of moxalactam alone and in combination with gentamycin against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother., 23, 179-181.
10. Moody, J. A., Gerding, D. N. and Peterson, L. R. (1987): Evaluation of ciprofloxacin's synergism with other agents by multiple in vitro methods. Am. J. Med., 82 (Suppl. 4A), 44-54.
11. Neu, H. C. (1989): Synergy of fluoroquinolones with other antimicrobial agents. Rev. Infect. Dis., 2 (Suppl. 5), S1025-S1035.
12. Madan, A., Dhar, A., Kulshrestha, P. P., Laghate, V. D. and Dhar, P. (1991): Preliminary observation on drug resistant cases of typhoid fever. J. Assoc. Physicians India, 39, 439-440.